REMARKS

In general, Applicant's invention features a method for C-terminal protein tagging. The method begins with a nucleic acid sequence that encodes the protein to be tagged. The nucleic acid is translated under conditions that allow stalling of translation at the 3' end of the protein coding sequence, thereby forming a stalled translation complex. To generate the tagged protein, this complex is contacted with a puromycin-tag under conditions that allow the puromycin-tag to be covalently bonded to the protein's C-terminus. The use of a stalled translation complex is an important aspect of Applicant's method. As noted in the present specification, for example, at page 12, line 14 - page 13, line 17, bonding of the puromycin-tag to the protein following translation stalling maximizes the yield of C-terminally tagged protein product.

Summary of the Office Action

Claims 1-31 are pending. Claims 1-13 are withdrawn from consideration, and claims 14-31 are under examination in this case. Claim 25 is rejected, under 35 U.S.C. § 112, second paragraph, as being indefinite. Claims 14-16, 19-26, and 28-31 stand rejected, under 35 U.S.C. § 102(e) as being anticipated by Yanagawa et al. (U.S. Patent No. 6,228,994 B1), and claim 27 stands rejected, under 35 U.S.C. § 103, as being unpatentable over a combination of Yanagawa et al. (U.S. Patent No. 6,228,994 B1) and Schatz et al. (U.S. Patent No. 5,723,584). Objections have also been raised to the drawings. Each of these issues is addressed below.

Support for the Amendments

Applicant has amended the specification to update the status of all cited U.S. patent applications and make grammatical corrections. In addition, claims 14, 15, 18, 20, 22, 24-26, and 28-30 have been amended for the purpose of clarity only. Figure 3 has been amended to correct a typographical error; this amendment finds support in the specification at page 5, lines 20-23, and page 9, line 24 to page 10, line 1. No new matter is added by any of these amendments, and the claims are not further limited.

Objection to the Drawings

In response to the objection to the drawings, Applicants submit herewith a set of formal figures in which all defects have been corrected. These figures satisfy 37 C.F.R §§ 1.84 and 1.152, and this objection may be withdrawn.

Rejection under 35 U.S.C. § 112, second paragraph

Claim 25 stands rejected, under 35 U.S.C. § 112, second paragraph, as being indefinite in the recitation of the phrase "wherein said first functional group has a reactivity orthogonal to the reactivity of said second functional group." This rejection is respectfully traversed as this language is standard and definite.

Those skilled in the art of chemistry and biochemistry routinely use such terminology to refer to two functional groups (as defined in the specification at page 4, lines 5-6) that have two different chemical reactivities. Applicant's specification makes

clear that this standard usage among chemists is the same usage intended here. For example, at page 8, lines 11-15, the specification states:

> Alternatively, the tag may be any functional group. Examples of useful functional groups include those with reactivities orthogonal to the reactivities of protein functional groups, for example, double bonds and ketones.

This passage indicates that useful functional groups for attachment as tags (i.e., the second functional group of claim 25) include double bonds and ketones, functional groups that differ in chemical reactivity from the functional groups of proteins (i.e., the first functional group of claim 25).

Applicant further notes that the Office, in applying a rejection of this claim for anticipation, also used the disputed claim term in its standard manner. In particular, at page 4, the Office Action stated (emphasis added):

> Regarding claim 25, the first function[al] group of said protein could be considered as any amino acid in said protein that had a different reactivity from said tag (second functional group such as biotin).

Thus, the Office also understood and applied this standard definition of Applicant's claim term. The indefiniteness rejection of claim 25, under 35 U.S.C. § 112, second paragraph, should be withdrawn.

Rejection under 35 U.S.C. § 102

Claims 14-16, 19-26, and 28-31 stand rejected, under 35 U.S.C. § 102, as being anticipated by Yanagawa et al. (U.S. Patent No. 6,228,994 B1). This rejection is respectfully traversed.

To support a *prima facie* case of unpatenability under § 102, a single prior art reference must describe all of the elements and limitations of the rejected claim. There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. *Scripps Clinic & Research Fdtn. v. Genentech, Inc.*, 927 F.2d 1565, 1576 (Fed. Cir. 1991). In the present case, the Office has not met this burden of proof because the method taught in the '994 patent differs in a fundamental respect from the method of claim 14. Claim 14 requires that *translation stalls at the 3' end of the nucleic acid sequence, forming a stalled translation complex comprising the protein* and that *the stalled translation complex is contacted with the puromycin-tag*. The '994 patent never teaches a method with these two steps.

In the Office Action (Paper No. 8), the Examiner concedes that "Yanagawa *et al.* did not directly show [ ] a stalled translation complex, as recited in step (b) of the claim" (Office Action, page 3). On this basis alone, the '994 patent cannont anticipate the claimed method.

Moreover, the Examiner, conceding that the '994 patent doesn't expressly teach a stalled translation complex, attempts to cure this deficiency with a fiction about the '994 teaching. Specifically, the Examiner states, "in the absence of convincing evidence to the contrary, a translation complex after the translation was terminated could be considered as a stalled translation complex." (Office Action, pages 3-4). This argument, however, fails because of both scientific and legal error.

Scientifically, this position is unsupportable. The process of translation is well understood. Immediately upon termination, the translation complex falls apart, with the two ribosomal subunits disengaging from the mRNA and releasing the protein (see attachment). Thus, normally there is no translation complex after translation terminates. The Office's suggested scenario simply doesn't exist under usual conditions.

The Office's position also fails to satisfy the legal burden to supply facts to support a conclusion of unpatentability. Where the PTO makes an unsupported assertion that a prior art device would function in a manner not explicitly disclosed, and the assertion is 'mere speculation unsupported by any rational basis for believing it might be true,' the burden does not shift to the applicants. *In re Donaldson Co. Inc.*, 29 USPQ2d 1845, 1852 (Fed. Cir. 1994). Here, the Examiner has not and cannot support the conclusion of anticipation because it rests on a precondition about translation complexes that does not exist under usual conditions.

Furthermore, the other claims included in the rejection under 35 U.S.C. § 102 are dependent from claim 14, and, as such, include all the limitations of claim 14. Therefore, the argument as stated above provides that these claims are also not anticipated by the prior art.

Even further to the above argument, regarding claim 29, Applicant points out that the claimed method requires the use of a tag that is a phenyl diboronic acid derivative. The Office argues that the prior art discloses a phenyl diboronic acid derivative, relying on Yanagawa Fig. 2 and the illustrated fluorthiopur compound. However, this compound is not

a phenyl diboronic acid derivative; indeed, fluorthiopur does not possess a boron at all.

For all of the above reasons, the § 102 rejection of claims 14-16, 19-26, and 28-31 should be withdrawn.

Rejection under 35 U.S.C. § 103

Claim 27 stands rejected, under 35 U.S.C. § 103, as being unpatentable over a combination of Yanagawa (U.S. Patent No 6,228,994 B1) and Schatz et al. (U.S. Patent No. 5,723,584). This rejection is also respectfully traversed.

With respect to the primary reference Yanagawa, the Office is directed to Applicant's discussion presented above. As indicated, Yanagawa does not disclose or suggest a method of C-terminal protein tagging that makes use of a stalled translation complex. In fact, the '994 patent teaches away from this step, teaching that the tag is introduced and attached during active protein synthesis. Moreover, Yanagawa's methods are limited to standard transcription/translation reactions using standard nucleic acid templates. Yanagawa does not suggest that the disclosed approaches for protein labeling might be improved, and certainly does not lead one skilled in the art to use a stalled translation complex in the protein labeling reaction.

The other cited reference, Schatz et al., does not provide what Yanagawa lacks. Schatz is limited to a disclosure of biotinylation peptides. Schatz does not discuss any method remotely related to Applicant's claimed method for C-terminal protein tagging, nor does it discuss the use of stalled translation complexes in any context.

Accordingly, even if these references were properly combined (which Applicant does not concede), the combination of Yanagawa and Schatz does not provide Applicant's claimed method for C-terminal protein tagging because neither reference discloses or suggests the important step of forming a stalled translation complex and contacting such a complex with the puromycin-tag. The § 103 rejection of claim 27 may be withdrawn.

Conclusion

Applicant submits that this case is now in condition for allowance, and such action is respectfully requested.

If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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F:\50036\50036.028002 Reply to Office Action

MARKED UP VERSION TO SHOW AMENDMENTS MADE

In the Specification:

Replace the first paragraph on page 1 (lines 5-6) with the following amended paragraph:

This application claims the benefit of the filing date of provisional application, U.S.S.N. 60/143,339, filed July 12, 1999, now abandoned.

Replace the first full paragraph on page 2 (lines 6-7) with the following amended paragraph:

In general, the invention features a protein having a covalently bonded puromycin-tag, the tag being positioned at the C-terminal end of the protein.

Replace the second full paragraph on page 2 (lines 8-16) with the following amended paragraph:

In preferred embodiments, the tag is a small molecule (for example, biotin); the tag is a detectable label (for example, fluorescein, rhodamine, or BODIPY, or derivatives thereof); the tag is a functional group (for example, a functional group having a reactivity orthogonal to the reactivity of one of the protein's functional groups); the tag is a tether for attachment to a solid support (for example, a column, bead, or chip); the tag is one member of a specific binding pair; the tag is a phenyl diboronic acid derivative; the puromycin-tag further includes a nucleotide sequence positioned between the tag and the puromycin; and the nucleotide sequence is between about 1-200 nucleotides in length.

Replace the third full paragraph on page 2 (lines 17-22) with the following amended paragraph:

In a related aspect, the invention features a method for C-terminal protein tagging, involving (a) providing a nucleic acid sequence encoding the protein; (b) translating the sequence under conditions in which translation stalls at the 3' end of the sequence, forming a stalled translation complex; and (c) contacting the stalled

amended paragraph:

Any appropriate type of ligation chemistry may be exploited to attach the tag to the puromycin moiety and, for example, to the 5'-hydroxy group of the puromycin. In a preferred embodiment of the invention, the puromycin-tags are synthesized using standard solid phase techniques, for example, as outlined in Figure 2. Commercially available phosphoramidites of biotin or fluorescein (Glen Research), for example, may be used to derivatize the 5'-terminus of puromycin or X_n-puromycin. These reactions may be carried out, for example, as described in Oligonucleotide Synthesis: A Practical Approach, ed. Gait, M.J. (IRL, Oxford).

Replace the first partial paragraph on page 9 (lines 1-14) with the following amended partial paragraph:

These derivatives include puromycin or X_n-puromycin linked to small molecules, for example, X_n-puromycin-5'-phosphate carrying a tethered biotin derivative (Figure 4); such a puromycin- or X_n-puromycin- tag may be used to attach a C-terminal biotin label to a protein, for example, for affinity purification. In a further example, a puromycin- or X_n-puromycin- tag may act as a bifunctional reagent by using a puromycin or X_n-puromycin derivative which contains both attachment and detection groups, for example, as shown in Figure 5. In this example, an X_n-puromycin derivative is tethered through its 5'-phosphate to both biotin and fluorescein moieties using standard oligonucleotide synthesis techniques. Attachment groups may include, without limitation, biotin, phenyl diboronic acid/salicylhydroxamic acid, 1,2-amino thiol, or ketone. Detection groups may include, without limitation, fluorescein or derivatives thereof, rhodamine or derivatives thereof, or BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene, Molecular Probes, Eugene, OR) or derivatives thereof.

Replace the paragraph beginning on page 9, line 24 and ending on page 10, line 5 with the following amended paragraph:

The scheme in Figure 3 outlines the synthesis of puromycin- or X_n-puromycin- tags which include a terminal modification that introduces a terminal amino or thiol functionality into the puromycin or X_n-puromycin intermediate. These reactions are again carried out as described above for Figure 2. As illustrated in Figure 7, these reactive moieties are then used to introduce non-protein functional groups, for example, a ketone, into the puromycin- or X_n-puromycin- tag.

Replace the first full paragraph on page 11 (lines 3-8) with the following amended paragraph:

In yet another embodiment, puromycin may be linked to polymers. In one particular example, puromycin may be attached to an oligonucleotide using previously described methods (Szostak et al., WO 98/31700; Szostak et al., U.S.S.N. 09/247,190 (1999), now U.S. Patent No. 6,261,804 B1; Roberts & Szostak (1997) Proc. Natl. Acad. Sci. USA 94:12297-12302) for the purpose of sequence-specific hybridization to a solid phase.

Replace the second full paragraph on page 11 (lines 9-14) with the following amended paragraph:

An appropriate puromycin- or X_n-puromycin- tag may also be used for the preparation of protein-protein conjugates. In one example, a puromycin or X_n-puromycin dimer (Figure 10) may be added to stalled ribosome complexes to generate protein homodimers. Puromycin or X_n-puromycin dimers are prepared as described above for Figure 2 using a second puromycin or puromycin derivative as a tag.

Replace the paragraph beginning on page 12, line 14 and ending on page 13, line 4 with the following amended paragraph:

To maximize the yield of the C-terminally tagged product, the tag is preferably attached to the full-length peptide or protein following translation of the open reading frame. This can be achieved by stalling the ribosome as an mRNA-ribosome-peptidyl complex after translation of the coding sequence. Ribosome stalling at the 3'-end of the open reading frame may be accomplished by any of a number of different methods. In one preferred approach, the message is engineered to be devoid of stop codons. As a result, release factors cannot bind, and the ribosome stalls (see, for example, Hanes & Plueckthun (1997) Proc. Natl. Acad. Sci. USA 94: 4937-4942). In another preferred approach, a DNA oligomer may be linked to the end of the message causing the ribosome to pause; this technique is described in Szostak et al., WO 98/31700; Szostak et al., U.S.S.N. 09/247,190 (1999), now U.S. Patent No. 6,261,804 B1; and Roberts & Szostak (1997) Proc. Natl. Acad. Sci. USA 94: 12297-12302). Alternatively, an in vitro translation lysate may be utilized which is devoid of release factors, as described in Lipovsek et al., Methods for Optimizing Cellular RNA-Protein Fusion Formation, U.S.S.N. 60/096,818; U.S.S.N. 09/374,962 (1999), now U.S. Patent No. 6,312,927; and WO 00/09737.

In the Claims:

Claims 14, 15, 18, 20, 22, 24-26, and 28-30 are amended as follows.

14. (Amended) A method for C-terminal protein tagging, comprising

(a) providing a nucleic acid sequence encoding [said] a protein;

(b) translating said nucleic acid sequence under conditions in which translation stalls at the 3' end of said nucleic acid sequence, forming a stalled translation complex comprising said protein; and

(c) contacting said stalled translation complex with a puromycin-tag under conditions in which said puromycin-tag is covalently bonded to the C-terminus of said protein.

15. (Amended) The method of claim 14, wherein [said] the tag of said puromycin-tag is attached to the 5'-hydroxy group of [said] the puromycin.

18. (Amended) The method of claim 14, wherein said [translation] translating step (b) is carried out in the substantial absence of at least one translation release factor.

20. (Amended) The method of claim 14, wherein [said] the tag of said puromycin-tag is a small molecule.

22. (Amended) The method of claim 14, wherein [said] the tag of said puromycin-tag is a detectable label.

24. (Amended) The method of claim 14, wherein [said] the tag of said puromycin-tag is a functional group.

25. (Amended) The method of claim 14, wherein said protein has a first functional group and [said] the tag of said puromycin-tag is a second functional group and wherein said first functional group has a reactivity orthogonal to the reactivity of said second functional group.

26. (Amended) The method of claim 14, wherein [said] the tag of said puromycin-tag is a tether for attachment to a solid support.

28. (Amended) The method of claim 14, wherein [said] the tag of said puromycin-tag is one member of a specific binding pair.

29. (Amended) The method of claim 28, wherein said [tag] one member is a phenyl diboronic acid derivative.

30. (Amended) The method of claim 14, wherein [said puromycin tag further comprises] a nucleotide sequence is positioned between [said] the tag and [said] the puromycin of said puromycin-tag.

MOLECULAR BIOLOGY OF THE CELL

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ATTACHMENT

make each new peptide bond. Two of these are required to charge each tRNA molecule with an amino acid (Figure 5–7). And two more drive two of the cyclic reactions occurring on the ribosome during synthesis itself: one for the aminoacyl-tRNA binding in step 1, and one for the ribosome translocation in step 3.

A Protein Chain Is Released from the Ribosome Whenever One of Three Different Termination Codons Is Reached[6,8]

As already noted, three of the codons in an mRNA molecule are **stop codons,** which terminate the translation process. A protein called *release factor* binds directly to any stop codon (UAA, UAG, or UGA) that reaches the A-site on the ribosome. This binding disturbs the activity of the nearby peptidyl transferase enzyme, causing it to catalyze the addition of a water molecule instead of the free amino group of an amino acid to the peptidyl-tRNA. As a result, the carboxyl end of the growing polypeptide chain is freed from its attachment to a tRNA molecule. Since it is only this attachment that normally holds the growing polypeptide to the ribosome, the completed protein chain is released into the cell cytoplasm (Figure 5–17).

The Initiation Process Sets the Reading Frame for Protein Synthesis[6,9]

In principle an RNA sequence can be decoded in any one of three different *reading frames,* each of which will specify a completely different polypeptide chain (see Figure 3–13, p. 108). Which of the three frames is actually read is determined when a ribosome engages with an mRNA molecule to form an *initiation complex.* This complex is assembled at the exact spot on the mRNA where the polypeptide chain is to begin.

The initiation process is complicated, involving a number of steps catalyzed by proteins called **initiation factors,** many of which are themselves composed of several polypeptide chains. Because of their complexity, many of the details of initiation are still uncertain. However, it is clear that each ribosome is assembled onto an mRNA chain as two separate subunits, the small ribosomal subunit being added first. Before the mRNA is bound, a special **initiator tRNA** molecule, recognizing the codon AUG and carrying methionine, is loaded onto each small subunit. This loading reaction is catalyzed by one of the initiation factors, called *initiation factor 2,* or *IF-2.* In some eucaryotic cells the overall rate of protein synthesis is controlled by this factor (see the following page).

The small ribosomal subunit binds to the region of the mRNA molecule where protein synthesis is to begin by pairing its bound initiator tRNA molecule with a particular AUG start codon (Figure 5–18). An mRNA molecule usually contains many AUG sequences, each of which codes for methionine. But the vast majority of these will not serve as start codons. As explained elsewhere, which AUG is recognized as a start codon depends on other parts of the mRNA nucleotide sequence (p. 332).

At the completion of the initiation process, all of the initiation factors associated with the small ribosomal subunit up to this point are discharged to make way for the binding of a large ribosomal subunit to the small one. In this way, a complete functional ribosome is formed. The initiator tRNA molecule ends up bound to the P-site of the ribosome, so that the synthesis of a protein chain can begin directly with the binding of a second aminoacyl-tRNA molecule to the A-site of the ribosome (Figure 5–18). Further steps in the elongation phase of protein synthesis then proceed as described previously (see step 2 of Figure 5–16).

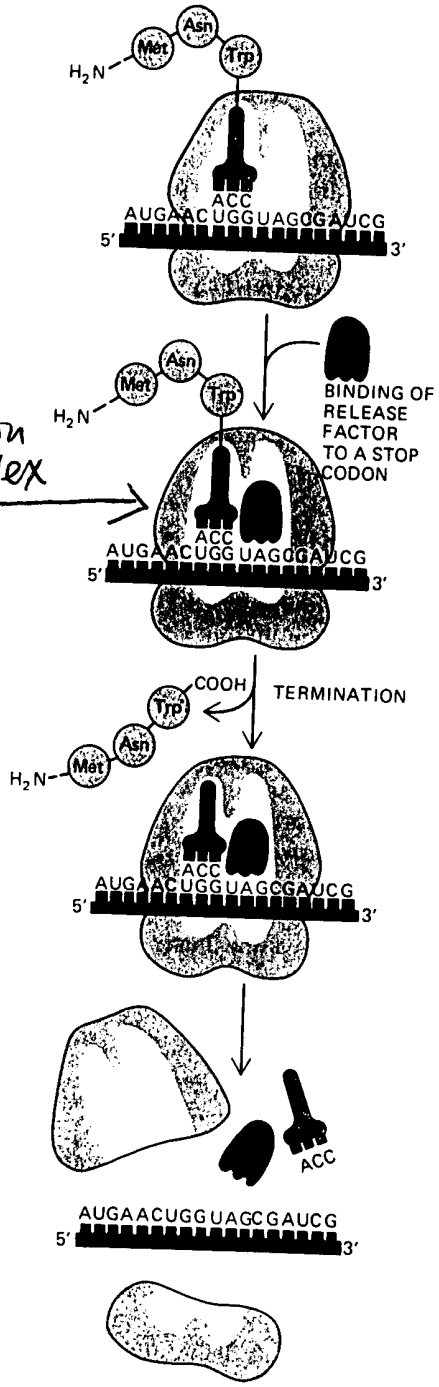

Figure 5–17 The final phase of protein synthesis. The binding of release factor to a stop codon terminates translation: the completed polypeptide is released, and the ribosome dissociates into its two separate subunits.

FIGURE 3 AMENDMENT

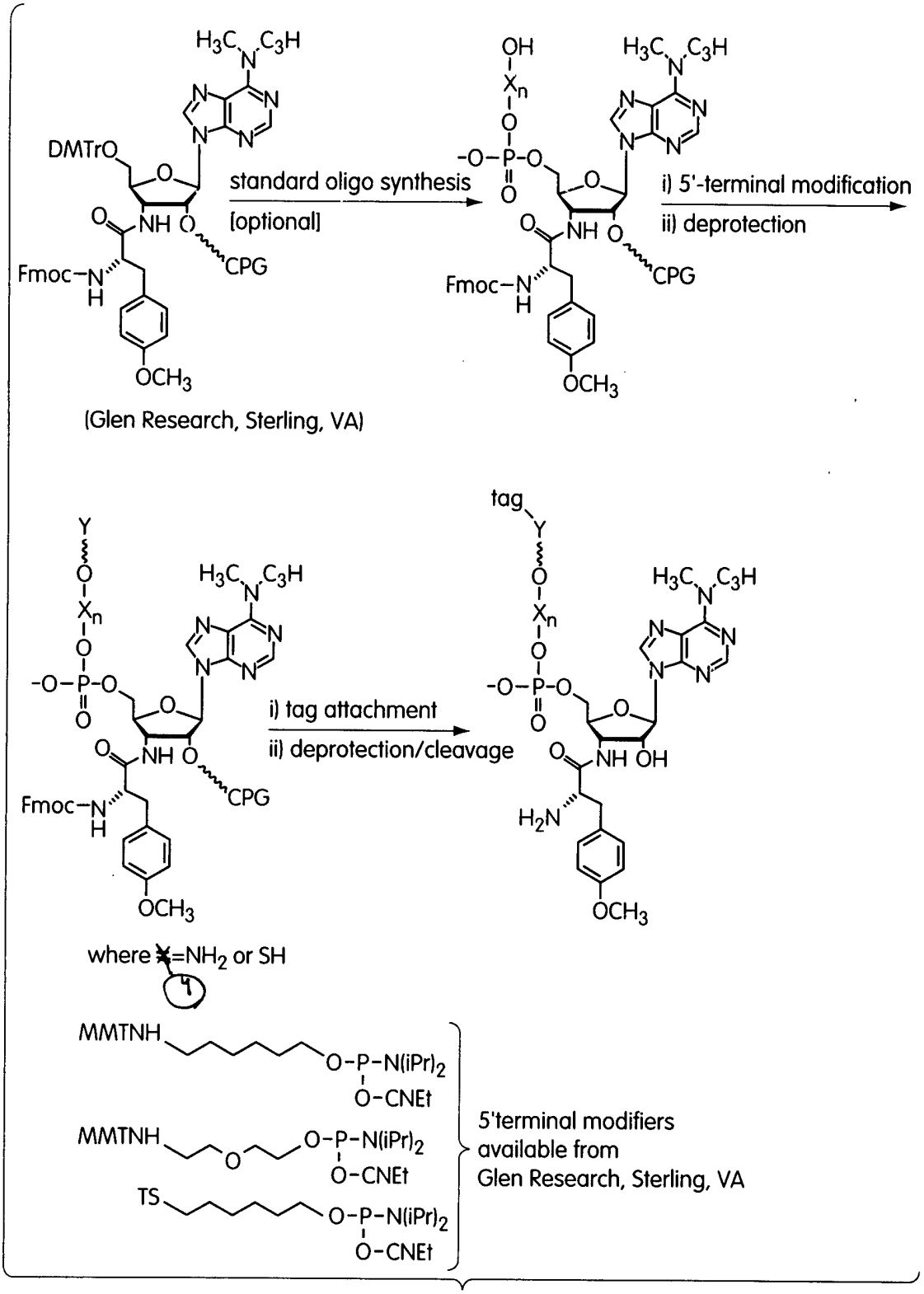

Fig. 3